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Exposure to anticoagulant rodenticides in steppe polecat (*Mustela eversmanii*) and European polecat (*Mustela putorius*) in central Europe

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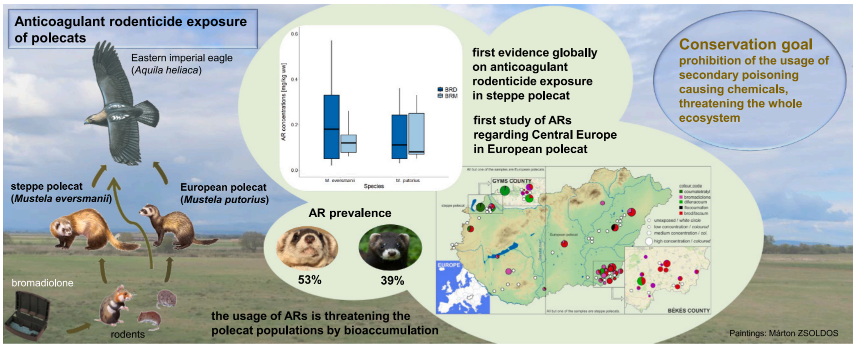
HIGHLIGHTS

- This study is the first evidence of anticoagulant rodenticide (AR) exposure in steppe polecat globally.
- ARs were first detected in European polecats in Central Europe.
- AR prevalence was 53% in steppe polecat and 39% in European polecat.
- AR accumulation increased with human effect and decreased with habitat naturalness.
- ARs threaten the food web; their prohibition or drastic reduction is proposed.

GRAPHICAL ABSTRACT



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ABSTRACT

Poisoning caused by coumarin-type anticoagulant rodenticides (ARs) stands as the predominant method for controlling rodents globally. ARs, through secondary poisoning, pose a significant threat to predators due to their lethal and sublethal effects. We examined the concentration of accumulated ARs in liver samples of mostly road-killed steppe polecats (*Mustela eversmanii*) and European polecats (*M. putorius*) collected throughout Hungary between 2005 and 2021. The steppe polecat samples were found mainly from Eastern Hungary, while European polecats from Western Hungary. We measured the concentration of six residues by HPLC-FLD. Our analysis revealed the presence of one first-generation and four second-generation ARs in 53% of the steppe polecat (36) and 39% of the European polecat (26) samples. In 17 samples we detected the presence of at least two AR compounds. Although we did not find significant variance in AR accumulation between the two species, steppe polecats displayed greater prevalence and maximum concentration of ARs, whereas European polecat samples exhibited a more diverse accumulation of these compounds. Brodifacoum and bromadiolone were the most prevalent ARs; the highest concentrations were 0.57 mg/kg and 0.33 mg/kg, respectively. The accumulation of

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ARs was positively correlated with human population density and negatively correlated with the extent of the more natural habitats in both species. To the best of our knowledge, this is the first study to demonstrate anticoagulant rodenticide exposure in steppe polecats globally, and for European polecats in Central European region. Although the extent of AR accumulation in European polecat in Hungary appears comparatively lower than in many other European countries, the issue of secondary poisoning remains a serious problem as these ARs intrude into food webs. Reduced and more prudent usage of pesticides would provide several benefits for wildlife, included humans. However, we advocate a prioritization of ecosystem services through the complete prohibition of the toxicants.

1. Introduction

Toxicants, accumulated in the environment lead to habitat degradation in such places thought to be pristine such as the Arctic, which also has miscellaneous harmful consequences (Bergmann et al., 2022). A portion of these toxicants penetrate into the food webs with bioaccumulation and biomagnification and exert their negative effects in the long term (e.g., dichlorodiphenyltrichloroethane - DDT (Kabasenche and Skinner, 2014)). Consequently, all living organisms are inevitably subjected to the resultant negative impacts (e.g., cobalt (Gál et al., 2008) or anticoagulant rodenticides (ARs) (Nakayama et al., 2019; Cooke et al., 2023; Keating et al., 2024)). Furthermore, the residues of these toxicants persist in our environment longer than other toxicants and can also be found in soil, freshwater and seawater (e.g., AR residues (Murphy, 2018)). Even ecosystem services vital to humans, such as the regulation of soil and water quality, are not immune to the damaging effects of accumulated toxicants (e.g., pesticides (Power, 2010)). Thus, if the accumulation of these toxicants is detectable in the environment or any living being in an ecosystem, we can conclude that the given toxicant is present throughout that ecosystem. Therefore, detecting a persistent toxicant can serve as an indicator of ecosystem health (e.g., pharmaceuticals (Zhang et al., 2021)). In primary poisoning animals, often including non-target species, directly consume the baits (Thomas et al., 2011; Sánchez-Barbudo et al., 2012). In secondary poisoning toxic substances transfer to non-target species through the food chain by consuming poisoned prey (Gál et al., 2008; Alomar et al., 2018). ARs, a type of toxicant prone to causing secondary poisonings (e.g., Winters et al., 2010; Sánchez-Barbudo et al., 2012; Nakayama et al., 2019; Deák et al., 2021), can lead to death of individuals even in very low concentrations (but this concentration cannot be defined exactly in wild-living species) and it has a negative impact on the consumer's fitness through sub-lethal effects (Knobel, 2015; Serieys et al., 2018; Sainsbury et al., 2018; Elmeros et al., 2019; Carrera et al., 2024; Keating et al., 2024).

Anticoagulant rodenticides (ARs) are generally used to control populations of terrestrial rodents, for example common voles (*Microtus arvalis*), rats (*Rattus* spp.), and mice (*Mus* spp.) that are considered pests in agricultural and urban ecosystems (Watt et al., 2005). The most commonly used ARs tend to accumulate in non-target species (Sánchez-Barbudo et al., 2012; Alomar et al., 2018). The modern second-generation ARs (SGAR) used mostly are considerably more effective than the former first-generation ARs (FGAR) which have moderate toxicity, as SGARs exhibit a considerably longer half-life and increased toxicity (Murphy, 2018). Their LD50 values typically range from 0.02 to 0.04 mg/kg, in contrast to the FGARs, which have LD50 values ranging from 10 to 50 mg/kg (Murphy, 2018).

Secondary exposure to ARs and secondary poisonings have been detected across a range of mustelid species, including the European polecat (*Mustela putorius*) (e.g., Shore et al., 2003; Fournier-Chambrillon et al., 2004; Baert et al., 2015; Elmeros et al., 2018; Sainsbury et al., 2018; Guldemond et al., 2020; Lestrade et al., 2021), least weasel (*Mustela nivalis*) (Sánchez-Barbudo et al., 2012; Fernandez-de-Simon et al., 2022), stoat (*Mustela erminea*) (McDonald et al., 1998; Elmeros et al., 2011), stone marten (*Martes foina*) (Berny et al., 1997; Sánchez-Barbudo et al., 2012; Elmeros et al., 2018), European pine marten (*Martes martes*) (Lestrade et al., 2021), European mink (*Mustela lutreola*) (Fournier-Chambrillon et al., 2004), American mink (*Neogale vison*) (Fournier-Chambrillon et al., 2004; Ruiz-Suárez et al., 2016), fisher (*Pekania pennanti*), and American marten (*Martes americana*) (Thomas et al., 2017).

We examined the exposure to ARs (i.e. FGARs: coumatetralyl, warfarin; SGARs: brodifacoum, bromadiolone, difenacoum, flocoumafen) of two closely related mustelids, the steppe polecat and the European polecat. The steppe polecat predominantly preys on the common vole, but from spring to summer (the offspring rearing period) it consumes common hamster (*Cricetus cricetus*) more frequently (Lanszki and Heltai, 2007), which is a critically endangered species globally (Banaszek et al., 2020). The European polecat primarily feeds on small rodents in Hungary (Lanszki and Heltai, 2007). Common vole and hamster are considered as agricultural pests which are targeted with ARs in Hungary, so rodent consumers are potentially exposed to these rodenticides. Despite regulatory restrictions implemented in Hungary in 2017 (coumatetralyl: Commission Implementing Regulation (CIR, EU) 2017/1378, warfarin: (CIR) 2017/1376, brodifacoum: (CIR) 2017/1381, bromadiolone: (CIR) 2017/1380, difenacoum: (CIR) 2017/1379, flocoumafen: (CIR) 2017/1383), the extensive use of ARs persists, posing a high risk of secondary poisoning for non-target species. However, regulations prohibit the use of these types of ARs in open areas, in a diffuse manner, and in high concentrations. The issue is that high concentrations are not expressed in kg/km^2. Instead, specific limits are defined for products or baits, for example: “products shall only be supplied with a maximum quantity of bait per pack of 50 g for grain, pellet or paste baits, and 100 g for wax block baits” (CIR 2017/1381). Consequently, farmers may purchase and use more packages or baits to compensate for the reduced AR content. For the European polecat, ARs have been previously identified, but Central Europe was a previously unexplored area regarding AR accumulation. However, these substances have not been previously tested in steppe polecat, and it is unknown whether secondary exposure occurs in this species.

Our research aimed to identify the presence and measure the concentrations of six coumarin-type ARs in both the steppe polecat and European polecat. We evaluated some potentially important correlates of exposure including demographic factors, effect of the season, condition, cause of death and spatial variables related to the accumulation of these toxicants. We hypothesized that ARs would be accumulated in both polecat species because AR use is widespread in the study area, and we anticipated differences in the accumulation of ARs between them due to their distinct land-use patterns and diet. Specifically, we predicted the steppe polecat, compared with the European polecat, would have greater exposure to ARs due to its stronger association with agrarian ecosystems, where pesticide use is more extensive.

2. Methods

2.1. Study species

Both studied polecat species are classified as “least concern” by the IUCN Red List; however, their status varies within their distribution ranges (Croose et al., 2018; Sainsbury et al., 2024). The steppe polecat occurs in Eurasia from Czech Republic to China and our study area (Hungary, Central Europe) is located at the western edge of its range. In

Europe, the steppe polecat exhibits small and fluctuating populations, with a notable decline in the number of individuals, especially in Eastern Europe, particularly Ukraine. Its situation in Asia is also considered to be unfavourable (Sainsbury et al., 2024). The species is protected in Hungary and exhibits a close ecological association with grasslands and agricultural areas, where its larger-size rodent prey species (hamster or ground squirrel (*Spermophilus citellus*)) is abundant. The European polecat is distributed from Spain to Russia, extending to the Ural Mountains (Skumatov et al., 2016) and Hungary is situated in the middle of its range. It has a large and relatively stable population overall, however there are large differences in its population trends from declining to increasing in its distribution area (Croose et al., 2018). European polecats can be legally harvested in Hungary except during the spring and early summer. Being a habitat generalist species, it inhabits wetlands, forests, agricultural areas and settlements. In addition to poisoning, both species are threatened by several other factors, including habitat loss, accidental road-killing, hybridization and pathogens (Croose et al., 2018; Szatmári et al., 2021; Lanszki et al., 2022).

2.2. Sampling

Over a 16-year period from 2005 through 2021, we examined steppe polecats and European polecats sampled across Hungary. We collected fresh carcasses of polecats that died within a day approximately, and stored them frozen at −20 °C. Additionally citizens and staff of national park directorates contributed to collecting polecat carcasses, which were also transported frozen to the Hungarian Natural History Museum (HNHM), and Kaposvár University Campus (KUC). Causes of mortality included vehicle collision (81%), harvesting (18%), and unknown causes (1%). We recorded the date and coordinates of collection for each polecat. Specimens were stored at the HNHM, KUC and the Balaton Limnological Research Institute.

During necropsy, we measured the body mass and other body dimensions (e.g., body length, tail length) and scored the body condition based on fat deposit over flanks between 1 (indicating poor condition) and 3 (good condition) (Simpson, 2000). Subsequently, the liver (and other tissues e.g., spleen, lungs, etc.) was dissected from the animals ($n = 63$) and preserved at −20 °C until the analytical measurement. It is noteworthy to highlight that ARs are recognized to remain stable during long-term frozen storage (Serieys et al., 2015). All analytical measurements were performed after the 16-year sampling period when all tissue samples were already collected. Furthermore, each of the polecats underwent thorough genomic identification processes because hybridization between the two species occurs (Szatmári et al., 2021) which ensured the accuracy and reliability of our taxonomy data. In addition these samples were also tested for canine distemper virus (Canine morbillivirus, CDV) (Lanszki et al., 2022).

2.3. Chemical analysis

The liver samples were analyzed between 2021 and 2022 at the National Food Chain Safety Office (NFCSO), Laboratory of Veterinary Diagnostic Directorate (VDD, Budapest, Hungary). The concentrations of FGAR (coumatetralyl, warfarin) and SGAR (brodifacoum, bromadiolone, difenacoum, flocoumafen) AR residues were measured. Both tissue extraction and analytical methods were implemented and optimized following the method of Chalermchaikit et al. (1993). High purity (>98%) chemicals and solvents were used to perform the chemical analysis: acetonitrile (ACN) (CAS# 75-05-8; HPLC Gradient Grade, Thermo Fisher Scientific, Hungary), methanol (MeOH) (CAS# 67-56-1; HPLC Plus Gradient Grade, Reanal, Hungary), acetic acid (CAS# 64-19-7; puriss. p.a., ACS reagent, reag. ISO, reag. Ph. Eur., ≥99.8%, Sigma Aldrich, Hungary), triethylamine (CAS# 121-44-8; ≥99.5% Sigma-Merck, Hungary), aluminium oxide (CAS# 1344-28-1; activated, basic, Brockmann I., for chromatographic use, Sigma-Merck, Hungary), ammonium acetate (CAS# 631–61-8; >98%, Sigma-Merck, Hungary). The following Pesticide Analytical Standard Grade (PESTANAL) analytical standards were purchased from Merck and used for detection and quantification: brodifacoum (CAS# 56073-10-0), bromadiolone (CAS# 28772-56-7), warfarin (CAS# 81-81-2), difenacoum (CAS# 56073-07-5, flocoumafen (CAS# 90035-08-8), and coumatetralyl (5836-29-3). Individual stock solutions were prepared in acetonitrile at a concentration of 1 mg/ml and stored in the dark at −20 °C.

Liver samples showing no signs of decomposition were used for the investigation. 2 g tissue samples were homogenized in 6 ml ACN by an electronic device (Grindomix, Retsch). After centrifugation, the supernatant was removed and saved. The pellet was extracted again with ACN and centrifugated. The collected supernatants were pooled and further purified by solid-phase extraction (SPE). The final SPE procedure was as follows. Initially, the Sep-Pak C18 (55–105 μm, 500 mg/6 ml, Waters, Hungary) column was conditioned with MeOH (4 ml) and equilibrated with H_2O (4 ml) followed by ACN (4 ml) at a flow rate of 2 ml min-1. Homogenized liver samples (2 ml) were passed through the Sep-Pak cartridge at a rate of 2 ml min^{-1}. Immediately following loading, cartridges were eluted with ACN (6 ml) at a flow rate of 2 ml min^{-1}. Extracts were evaporated to dryness by nitrogen gas stream (55 °C, 4–12 psi) and reconstituted with MeOH (150 μl) and H_2O (50 μl) induced by ultrasound and vortex mixing. Solvents and additives to SPE was all of HPLC quality and purchased from Reanal (Hungary). High-performance liquid chromatography with fluorescence detection (HPLC-FLD) was performed with an Agilent Technologies 1260 Infinity chromatograph consisting of a G1311B quaternary pump, a G1321 fluorescence detector and Agilent manual injector unit. 20 μl of purified sample extract were injected onto the reversed-phase analytical column (Supelcosil LC-18, 25 cm × 4.6 mm, 5 μm, Merck, Hungary). The chromatographic conditions were the same as described in the Chalermchaikit et al. (1993) study. The detection of the six ARs was performed simultaneously (Appendix A, Fig. A1–3). The fluorescent detector was set at an excitation wavelength of 318 nm and an emission wavelength of 390 nm. All measurements were performed on the same columns under the same chromatographic conditions. The data collection and processing were performed using Agilent ChemStation (Chemstation A.08.03) software package.

For quantitative analysis, five-point matrix-matched calibration curves were used for each external standard. For this, chicken liver tissue was used to ensure that the matrix did not contain ARs (Appendix A, Fig. A4). All analytical parameters for detection and quantification (e.g., LOD, LOQ, linearity, calibration ranges) are included in Appendix A, Table A2. The calculated concentration data are expressed as mg/kg (wet mass). A representative chromatogram showing the presence of ARs in a collected tissue sample is included in Appendix A, Figs. A5–7.

2.4. Statistical analysis

In our study, dataset of 62 individuals were included in the statistical models, comprising 36 steppe polecats and 26 European polecats. One hybrid individual ($n = 1$) was excluded from the analysis. We calculated the prevalence (the percent of individuals exposed to the given compound), mean, median, standard deviation (SD) and the range of concentration values by AR-types by species. These values were calculated in case of the concentrations of the total AR (ΣAR) and also were calculated concerning those individuals which contained any accumulated ARs (ΣAR_without0). An independent-samples *t*-test was conducted to compare the maximum AR concentrations by AR-types between the two species. Additionally, we investigated factors (Table 1) that might influence the concentrations of ΣAR, brodifacoum and bromadiolone dependent variables (Table 2) in the individuals through the application of multivariate linear models (LM). The other ARs were not included in further analysis due to limited sample sizes. Samples without any AR residue were included in the analyses. The residuals of dependent variables used in these models did not exhibit a normal distribution, probably due to the small number of elements, but were closest to this distribution based on the Q-Q plot. For the dependent variable representing the number of accumulated ARs in each individual (NoAR), associations were analyzed using ordinal logistic regression, while binary logistic regression was employed for the prevalence in individuals (whether an individual contained any AR) dependent variable (Table 2). Those samples which did not contain any AR residues were also included in these analyses. In cases where data on independent variables were missing for certain individuals, those individuals were excluded from the statistical tests. We created models to continuous dependent variables and prevalence in individuals with MASS package (Venables and Ripley, 2002) and to NoAR with ordinal package (Christensen, 2023). We ordered the combination of independent variables using dredge function (MuMIn package (Barton, 2020)), employing Akaike's Information Criterion (AIC) within each model. From these ordered combinations, variables of the final model, where ΔAIC was the lowest (in our models 0.00), were selected using get.models function (MuMIn package). Two types of condition estimation methods were utilized in the models: the body condition and the scaled mass index (SMI). SMI based on body mass and total body length (body length + tail length) using the formula $\text{SMI} = M_i[L_0/L_i]^{\text{bSMA}}$, where M_i is the body mass, L_i is the length of individual i, L_0 is the mean of body length of the sample and bSMA is the scaling exponent (Peig and Green, 2009; Peig and Green, 2010). The exponent values for the steppe polecat population and European polecat population were 0.23 and 0.26, respectively. We included them together into the models because there was no correlation between them in either species (Spearman's rank-order correlation, $r = 0.263$, $p = 0.132$ in steppe polecat, $r = 0.301$, $p = 0.197$ in European polecat). Spatial independent variables included data on the human population density. To preparing this, we determined which settlement lay within the administrative boundaries where the sample was collected. Subsequently, we retrieved human population density data for this settlement from the Hungarian Central Statistical Office (KSH) database (https://www.ksh.hu) for the collection year. Additionally, the Golden Crown value (Popp and Stauder, 2003; Tóth-Naár et al., 2018) indicating combined land productivity and profitability, obtained from the KSH, was also included among our independent spatial variables. We coded the Golden Crown values as ordinal categories. Habitat information were extracted from the Corine Land Cover (CLC, 2018) map database (European Environment Agency 2018). CLC classes, as detailed by Kosztra et al. (2019), were employed in Quantum GIS version 3.10 (QGIS Development Team 2019). To determine the extent of the five main habitat types (Table 1), based on the average home range sizes of the two polecat species (the mean home range size of males (318.6 ha ± 261.8 ha) measured was greater than that of females (221.4 ha ± 92.2 ha) in steppe polecat (Ottlecz, 2010; Ottlecz, 2012) and mean male home ranges were also significantly larger than those of females (820.2 ha ±164.8, $n = 12$, and 155.1 ha +/− 41.3, $n = 6$) in European polecat) (Rondinini et al., 2006; Ottlecz, 2010; Ottlecz, 2012), we used a 2-km radius (12.56 km^2) around the collection site and we measured the distance of the collection site from the nearest settlement. We examined the habitat differences between the two species with PERMANOVA. Additionally, we assessed differences in collection sites and human population density within the administrative boundaries to which the collection sites belong using the Mann-Whitney U test.

Table 1

The independent variables including Corine Land Cover (CLC) codes for habitat descriptors for linear models of the concentrations of the total AR (ΣAR), brodifacoum and bromadiolone residues in polecat liver samples collected in Hungary and for dependency analysis of the number of accumulated ARs (NoAR) and prevalence in individuals. Abbreviations: SP = steppe polecat, EP = European polecat. Variables marked with* only were used in the models of steppe polecat, while those marked with † was used for the European polecat because of the uneven number of elements in groups.

Variable	Type	Groups	Description
Sex*	Nominal	1	Male ($n = 22$)
		2	Female ($n = 13$)
Age*	Ordinal	1	Subadult (n = 6)
		2	Adult ($n = 28$)
Season	Nominal	1	Spring+summer (SP, $n = 19$; EP, n = 6)
		2	Autumn+winter (SP, $n = 9$; EP, n = 20)
SMI	Scale	–	The SMI of individuals (SP, $n = 35$; EP, $n = 20$)
Body condition	Ordinal	1	≤ 2.6 on the estimated scale from 1 to 3 (SP, n = 12; EP, n = 12)
		2	≥ 2.7 on the estimated scale from 1 to 3 (SP, n = 22; EP, $n = 8$)
Cause of death†	Nominal	1	Road-killed ($n = 18$)
		2	Trapped ($n = 7$)
Year	Ordinal	–	The year of sample collection
Human population density	Scale	–	The human population density in the nearest settlement of the collection site of the given sample in the collection year [person/km^2]
Distance	Scale	–	The distance of the closest settlement from the collection site [m]
Arable land	Scale	–	Area of Arable land (CLC 2.1 + 2.4.2) [ha]
Wetland	Scale	–	Area of Water bodies (CLC 5) + Inland wetlands (CLC 4.1) [ha]
Forest	Scale	–	Area of Forest (CLC 3.1) [ha]
Grassland	Scale	–	Area of Natural grassland (CLC 3.2.1) + Pastures (CLC 2.3.1) [ha]
Artificial surfaces	Scale	–	Area of Artificial Surfaces (CLC 1) [ha]
Golden Crown value	Ordinal	–	The Golden Crown value is a measurement unit of the quality of agricultural land in Hungary

Table 2

The description of the dependent variables for the modelling of influencing factors of accumulation of ARs in the steppe polecat and the European polecat. BRD: brodifacoum, BRM: bromadiolone, NoAR: number of accumulated ARs.

Variable	Type	Groups	Description
ΣAR	Scale	–	The Σ of accumulated AR concentration by individuals, included 0 values
BRD	Scale	–	The concentration of brodifacoum by individuals, included 0 values
BRM	Scale	–	The concentration of bromadiolone by individuals, included 0 values
NoAR	Ordinal	0	Number of individuals which contained no AR ($n = 33$)
		1	Number of individuals which contained 1 type of AR (n = 12)
		2	Number of individuals which contained 2 or more type of ARs ($n = 17$)
Prevalence in individuals	Nominal	0	Those individuals which contained no AR (n = 33)
		1	Those individuals which contained at least one AR ($n = 29$)

The relationship between NoAR and the continuous dependent variables, the concentration of ΣAR, brodifacoum and bromadiolone were examined with Spearman's rank-order correlation by species. Samples that did not contain any AR residue were also included in these statistics.

Statistical analyses were performed in IBM® SPSS® 29, RStudio 4.3.2 (Team, 2020) and PAST 3.20 (Hammer and Harper, 2001).

3. Results

3.1. Spatial distribution of samples and accumulated AR residues

Our spatial analysis revealed distinct patterns in the distribution of the two polecat species and their accumulated AR residues (Fig. 1). Samples collected east of the Danube river, Great Hungarian Plain predominantly consisted of steppe polecats, with only two exceptions, while samples west of the Danube were primarily European polecats, with only one exception.

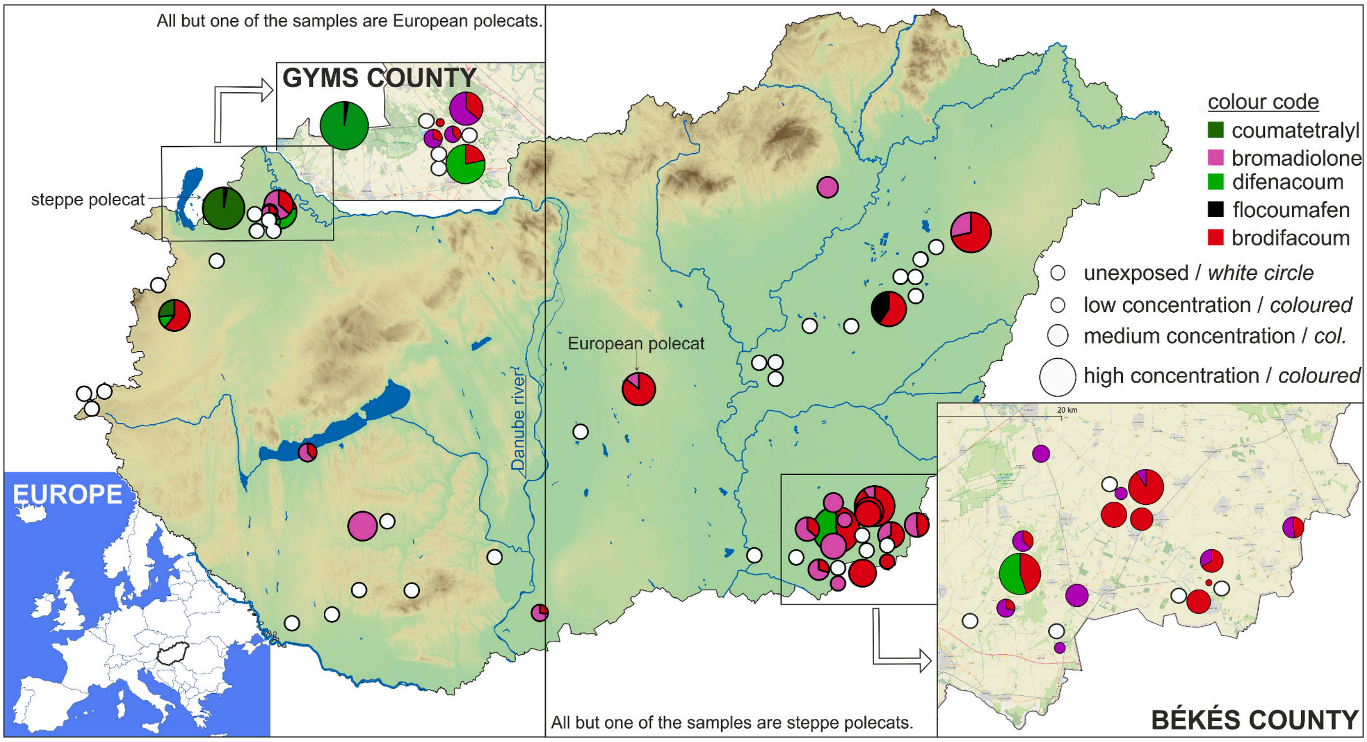

Fig. 1. Spatial distribution of the collected polecats in Hungary, highlighting the types and concentrations of detected AR residues. Circles without color indicate the absence of AR accumulation in liver samples, while the size of colored circles is directly proportional to the concentrations of the accumulated ARs. Areas with clustered samples are enlarged for better visibility.

3.2. Prevalence and concentration of ARs

We analyzed data of 36 steppe polecat, 26 European polecat and one *Mustela putorius* x *eversmanii* hybrid. We detected the presence of one FGAR (coumatetralyl) and four SGAR (brodifacoum, bromadiolone, difenacoum, flocoumafen) residues. 53% was the prevalence (19 individuals) of the liver samples examined in steppe polecat and 39% (10 individuals) in European polecat (Table 3, Appendix A, Table A1). Warfarin was not detected in any of the individuals. From the AR-positive individuals, brodifacoum (13 steppe polecat individuals, 8 European polecat individuals) and bromadiolone (12 steppe polecat individuals, 7 European polecat individuals) were the most prevalent ARs in both species (Table 3, Fig. 2); 10 steppe polecats (28%) and two European polecats (8%) contained one compound, nine steppe polecats (25%) and seven European polecats (27%) exhibited the presence of two types of ARs, and one European polecat contained three types AR residues (4%).

The distribution of concentrations varied among the different types

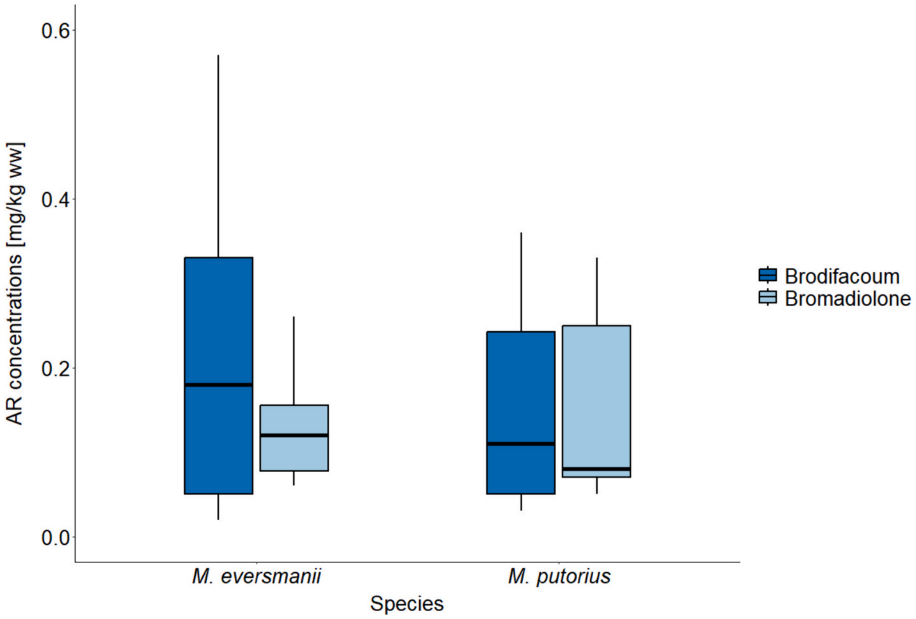

Fig. 2. Concentrations of the most prevalent AR residues by polecat species in Hungary. BRD: brodifacoum, BRM: bromadiolone.

Table 3
Summary dataset of concentrations of the total anticoagulant rodenticides (ΣAR) and individual active substances of ARs in polecat species. COU: coumatetralyl, BRD: brodifacoum, BRM: bromadiolone, DFC: difenacoum, FLO: flocoumafen, NumAR: number of accumulated AR residues in samples.

	Substance	Prevalence [%]	NumAR	Mean [mg/kg]	Median [mg/kg]	SD [mg/kg]	Max [mg/kg]	Min [mg/kg]
Steppe polecat	ΣAR	53	19	0.15	0.02	0.23	0.87	0.02
	ΣAR_without0	100	19	0.29	0.23	0.24	0.87	0.02
	COU	3	1	0.67	0.67	–	0.67	0.67
	BRD	36	13	0.21	0.18	0.18	0.57	0.02
	BRM	33	12	0.13	0.12	0.06	0.26	0.06
	DFC	3	1	0.48	0.48	–	0.48	0.48
	FLO	3	1	0.02	0.02	–	0.02	0.02
European polecat	ΣAR	39	10	0.12	0.00	0.17	0.47	0.10
	ΣAR_without0	100	10	0.30	0.33	0.14	0.47	0.10
	COU	4	1	0.10	0.10	–	0.10	0.10
	BRD	31	8	0.15	0.11	0.12	0.36	0.03
	BRM	27	7	0.16	0.08	0.12	0.33	0.05
	DFC	8	2	0.21	0.21	0.22	0.36	0.05
	FLO	4	1	0.19	0.19	–	0.19	0.19

of AR residues (Appendix A, Table A1). The highest concentration of accumulated SGAR residue was 0.57 mg/kg for brodifacoum in a steppe polecat, while the ΣAR ranged from 0.02 to 0.87 mg/kg in steppe polecat and from 0.10 to 0.47 mg/kg in European polecat (Appendix A, Table A1).

3.3. *Differences in AR accumulation and spatial characteristics between the two polecat species*

There was no significant difference between the prevalence in steppe polecats (53%) and in European polecats (39%). However, the European polecat tended to be more frequently contaminated by more than one compound, as this was observed in 31% of the samples (number of compounds, $\bar{x} = 1.90$, SD = 0.57, median (M) = 2). One European polecat contained three different types of ARs. In contrast, exposure to multiple compounds was less frequent in steppe polecats ($\bar{x} = 1.47$, SD = 0.51, M = 1), and occurred in 25% of samples. While steppe polecats exhibited a higher maximum concentration of AR residues compared to European polecats ($\bar{x} = 0.40$ mg/kg vs. 0.27 mg/kg), this difference was not statistically significant ($t = 1.032$, $p = 0.332$). However, in three out of the five cases (COU, BRD, DFC), the maximum concentration of AR residues and ΣAR found was higher in steppe polecats than in European polecats. No ARs were detected in the hybrid individual.

The coverage of each habitat types within a 2-km radius around

Table 4
The results of models related to AR exposure of steppe polecat. Bolded p-values indicate the significant effect of the variable.

Model	Variable	Estimate	Std. error	t value	p
ΣAR	Intercept	0.967	0.410	2.359	0.028
	Grassland	−0.001	0.001	−1.619	0.119
	Age	0.244	0.126	1.930	0.067
	Sex	**−0.454**	**0.166**	**−2.738**	**0.012**
	Population density	**0.001**	**<0.001**	**2.396**	**0.026**
	SMI	**−0.001**	**<0.001**	**−2.861**	**0.009**
BRD	Intercept	−84.490	54.62	−1.547	0.138
	Year	0.042	0.027	1.556	0.135
	Grassland	**−0.001**	**<0.001**	**−2.995**	**0.007**
	Age	**0.304**	**0.078**	**3.924**	**0.001**
	Sex	**−0.389**	**0.099**	**−3.930**	**0.001**
	Population density	**0.001**	**<0.001**	**4.437**	**<0.001**
	SMI	**<−0.001**	**<0.001**	**−4.098**	**0.001**
	Distance	<0.001	<0.001	1.873	0.076
BRM	Intercept	83.310	23.76	3.507	0.002
	Year	**−0.041**	**0.012**	**−3.513**	**0.002**
	Sex	**0.112**	**0.040**	**2.804**	**0.010**
	SMI	**<0.001**	**<0.001**	**2.084**	**0.048**
NoAR	Year	−1424.118	864.572	−1.647	0.100
	Age	**6.054**	**2.903**	**2.085**	**0.037**
	Artificial surfaces	−0.939	0.503	−1.867	0.062
	SMI	**2.047**	**0.970**	**2.110**	**0.035**
	Wetland	**−1.237**	**0.625**	**−1.979**	**0.048**
Prevalence in individuals	Intercept	−5,182,000	405,000,000	−0.013	0.990
	Golden Crown value	−1392	107,600	−0.013	0.990
	Season	4.132	321,700	0.013	0.990
	Forest	225.9	17,700	0.013	0.990
	Year	2579	201,600	0.013	0.990
	Grassland	−31.140	2420	−0.013	0.990
	Condition	−6735	527,600	−0.013	0.990
	Age	13,880	1,085,000	0.013	0.990
	Artificial surfaces	−31.120	2433	−0.013	0.990
	Sex	−4128	324,600	−0.013	0.990
	Population density	16.68	1305	0.013	0.990
	SMI	−8.429	658.8	−0.013	0.990
	Arable land	−19.55	1524	−0.013	0.990
	Wetland	−161.400	12,620	−0.013	0.990

collection sites difference between the two species (non-normal data distributions, one-way PERMANOVA, Bray–Curtis dissimilarity matrix, $F = 21.59$, $p = 0.0001$). By SIMPER analysis (Bray–Curtis dissimilarity matrix), arable lands accounted for 50.7% and forest 23.9% of this difference, with steppe polecat sites having more arable land and European polecat sites having more forest; Appendix B. Additionally, European polecat collection sites were closer to the nearest settlement compared to those of steppe polecats (non-normal data distributions, Mann-Whitney U test, mean ± SE, steppe polecat 2062 ± 290 m, European polecat 593 ± 133 m, $U = 147$, $n_1 = 31$, $n_2 = 25$, $z = 3.96$, $p = 0.0001$). However the human population density (mean ± SE, persons/km^2) around the collection site did not differ significantly between the two polecat species (Mann-Whitney U test, steppe polecat 77.8 ± 19.6, European polecat 91.0 ± 22.2, $U = 343.5$, $n_1 = 33$, $n_2 = 25$, $z = 1.08$, $p = 0.284$).

3.4. *Influencing factors of AR residue accumulation*

The ΣAR concentration values in steppe polecat were associated with higher human population density and lower SMI, and were higher in males (Table 4). The male steppe polecats and individuals with lower SMI and where the human population density was higher were also contained brodifacoum in higher concentrations. Adult age positively associated with brodifacoum concentration, while grassland area was negatively correlated with it. In contrast, bromadiolone concentrations were higher in females and individuals with higher SMI. Higher bromadiolone concentration was found in samples collected in earlier years. NoAR was higher in adult individuals and those with higher SMI, but lower in areas with more wetland. Prevalence in individuals was not associated with any examined independent variable.

In European polecats, the ΣAR and brodifacoum concentrations were negatively associated with forest area, and brodifacoum concentration was also negatively associated with grassland area (Table 5). The bromadiolone concentration was higher where the population density was higher. NoAR and prevalence in individuals showed no relationship with any examined variables.

NoAR increased with ΣAR, brodifacoum and bromadiolone concentration in both species.

4. Discussion

4.1. *Prevalence and concentrations in Hungary and Europe*

Our study revealed that 46% of examined polecat individuals, as we hypothesized, were exposed to AR at sub-lethal levels at least once in their life. Despite our hypothesis, no statistically significant difference in AR prevalence was observed between polecat species (Table 3, Supplementary material 1, Tables S1.1–2). However, the slight difference noted may still suggest a potentially elevated risk of exposure for the steppe polecat. Therefore, further studies are needed to assess the actual level of risk across the broader distribution of this species. It is important to note that the actual prevalence of AR exposure may be higher than our findings suggest, as finding poisoned individuals can be challenging. Those individuals that succumbed to lethal doses of ARs would not typically be found among road-killed specimens (Shore et al., 1999), because they often perish in remote areas and inaccessible locations (Birks, 1998). Using detection dogs can be a considerable help in finding poisoned individuals and baits (Deák et al., 2021). In addition to often time-consuming and costly field research, modelling techniques can also be valuable tools for investigating poisonings (Topping and Elmeros, 2016).

Our results indicated that brodifacoum and bromadiolone residues were the most frequently detected ARs, which aligns with international trends. Brodifacoum (31%) was closely followed by bromadiolone at 30%, and difenacoum at 26% in the livers of non-target species worldwide between 1998 and 2015 (Nakayama et al., 2019). The detection of these compounds can also be attributed to several factors as absorption, distribution, metabolism, and excretion (Horak et al., 2018). The prevalence of brodifacoum at the top of the list may be attributed to its longer half-life compared to other ARs (Nakayama et al., 2019). The relatively low concentration and prevalence of flocoumafen maybe due to its composition, as it contains a higher proportion of the less persistent diastereomer compared to other SGARs (Damin-pernik et al., 2017). Similarly, coumatetralyl's low prevalence in the samples can be attributed to that fact that this is a FGAR with shorter half-lives (Vandenbroucke et al., 2008). However, it is important to note that half-lives can vary among different species (Campbell et al., 2024). There can be regional variations in AR accumulation: for example, difenacoum exhibited a higher prevalence than brodifacoum in Britain (Shore et al., 2003). We did not detect warfarin, likely due to the absence of legally

Table 5

The results of models related to AR exposure of European polecat. Bolded *p*-values indicate the significant effect of the variable.

Model	Variable	Estimate	Std. error	t value	p
ΣAR	Intercept	0.663	0.253	2.616	0.020
	Forest	**−0.001**	**<0.001**	**−2.437**	**0.029**
	Grassland	−0.001	<0.001	−1.863	0.084
	Arable land	<−0.001	<0.001	−1.569	0.139
BRD	Intercept	0.503	0.183	2.751	0.017
	Forest	**−0.001**	**0.000**	**−2.420**	**0.031**
	Grassland	**−0.001**	**0.000**	**−2.408**	**0.032**
	Population density	−0.000	0.000	−1.694	0.114
	Arable land	−0.000	0.000	−2.002	0.067
BRM	Intercept	0.004	0.023	0.165	0.871
	Population density	**0.001**	**0.000**	**3.907**	**0.001**
NoAR	Season	−210.093	2113.691	−0.099	0.921
	Forest	−286.690	700.947	−0.409	0.683
	Grassland	−104.903	1832.528	−0.057	0.954
	Cause of death	64.178	3821.710	0.017	0.987
	Artificial surfaces	−130.533	4597.820	−0.028	0.977
	Population density	17.3494	3344.155	0.005	0.996
	Arable land	−702.838	961.087	−0.731	0.465
Prevalence in individuals	Intercept	−459.367	142,203.685	−0.003	0.997
	Golden Crown value	108.451	30,992.040	0.003	0.997
	Condition	−213.004	62,942.776	−0.003	0.997
	Population density	7.357	2098.518	0.004	0.997
	Distance	−0.372	105.616	−0.004	0.997
	Wetland	3.454	979.335	0.004	0.997

available rodenticide containing warfarin in Hungary.

A geographical pattern is evident in the data on prevalence for the European polecat (Fig. 3, Supplementary material 1, Table S1.1). In Denmark, AR residues were nearly ubiquitous, approaching 100% (Elmeros et al., 2018), whereas in France, only 15% of specimens exhibited AR accumulation (Fournier-Chambrillon et al., 2004; Lestrade et al., 2021). Differences in land use and the density of buildings (farms) in rural areas may explain the exceptionally high prevalence in some regions of Denmark (Elmeros et al., 2018) compared to France. In France, a portion of the samples was collected from more natural places, such as a protected mountain area, which likely contributed to the lower prevalence. Denmark responded to these high prevalence rates by implementing stricter regulatory restrictions; however, these measures did not reduce the prevalence of ARs. In fact, both the prevalence and AR concentrations increased in European polecat and stone marten after the regulatory changes (Elmeros et al., 2018), and this occurred in relation to red fox (*Vulpes vulpes*) in the UK (Campbell et al., 2024) and several other species in Finland (Koivisto et al., 2018) as well. This increase was attributed to the continued use of ARs in urban areas, which was linked to the AR concentrations in mustelids (Elmeros et al., 2018). According to the last review, Denmark had the highest prevalence of ARs among all examined species, at 93% (Nakayama et al., 2019). Globally, the real prevalence resulting from legal and illegal poisonings can be much higher than the detected values, as demonstrated by a study where simulated baits were used in a large-scale field experiment modelling various factors (Olea et al., 2022). Besides spatial patterns, temporal trends also represent important information about AR accumulation (Supplementary material 1, Table S1.1.); for instance, AR concentrations in Britain increased by a factor of 1.7 from 1992 to 2016 (Sainsbury et al., 2018). However due to insufficient temporal data on European polecats from the same countries, we cannot draw far-reaching conclusions, emphasizing the need for long-term studies. Increasing AR exposure has also been detected in other species such as fisher (Gabriel et al., 2015), bobcat (*Lynx rufus*) (Serieys et al., 2015) and grey wolf (*Canis lupus*) (Musto et al., 2024). In contrast, our study found a decreasing trend in bromadiolone concentration in steppe polecats.

Bromadiolone was detected in the highest concentration, followed by brodifacoum and difenacoum (Supplementary material 1, Fig. S1.1). The usage of SGARs is characteristic nowadays in contrast to FGARs; warfarin was not detected in any of the European polecats.

The measured concentrations in Hungary were comparatively lower than those observed in many other European countries (Supplementary material 1, Fig. S1.2, Table S1.1). In most countries, the parallel usage of more types of ARs is characteristic, however, in France, only bromadiolone was accumulated but it was in a very high concentration (Fig. S1.2).

4.2. *Differences in AR accumulation and spatial distribution of samples between the two polecat species*

Several factors may contribute to the variations observed in absorption and concentration values in samples of the two polecat species. The primary cause could be interspecific differences in habitat utilization. Steppe polecats primarily inhabit agricultural areas (Sainsbury et al., 2024, Appendix B), where farmers tend to use only a few type of ARs in high quantities against hamsters and voles. In contrast, European

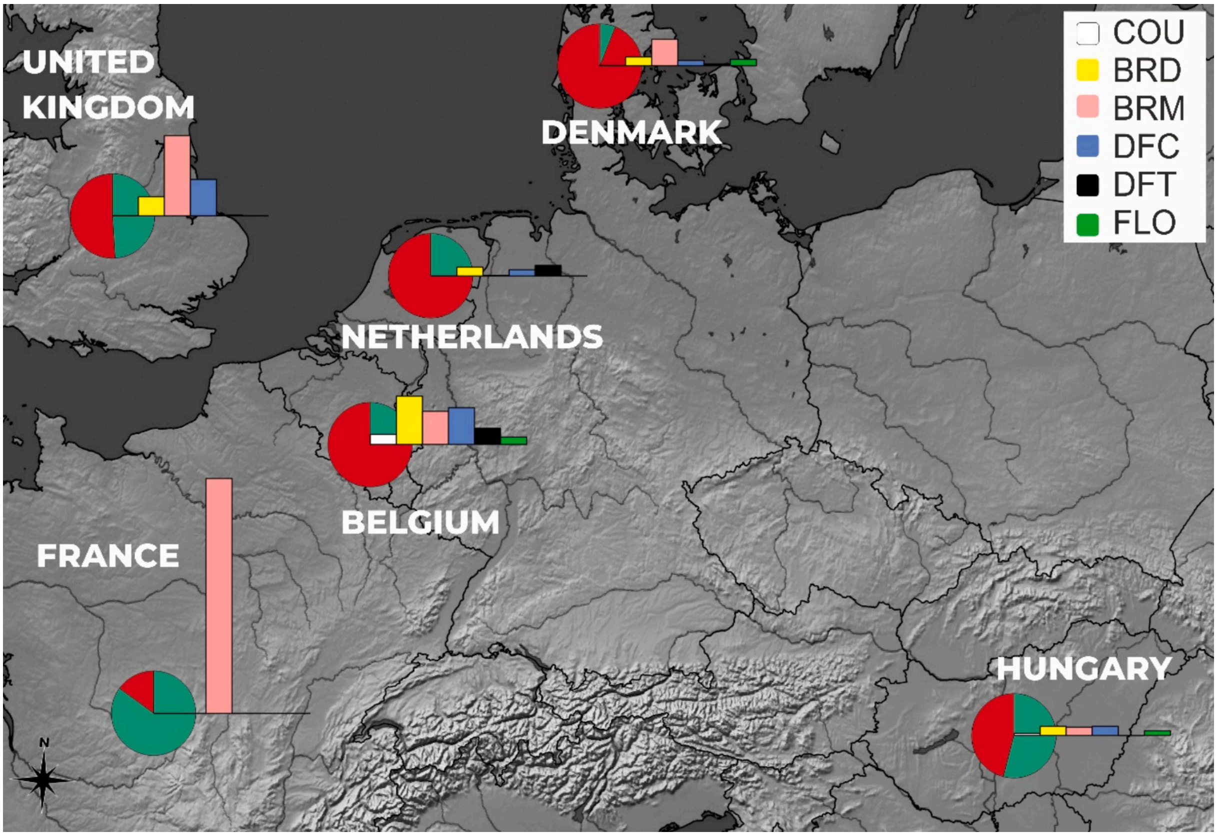

Fig. 3. Prevalence (pie charts) and concentrations (barplots) of ARs detected so far in European polecats by countries. The circles show the prevalence, where the red color means the proportion of samples with accumulated AR residues, while the green color means the proportion of samples without accumulated AR residues in the given country. The barplots show the concentration of accumulated AR residues by AR types by countries (Supplementary material 1). COU: coumatetralyl, BRD: brodifacoum, BRM: bromadiolone, DFC: difenacoum, FLO: flocoumafen. AR residues in European polecats were also investigated in other countries including Italy, Scotland, and Spain. However, due to the limited sample sizes (only 1–2 samples), these results are not presented here but are available in the Supplementary material 1, Table S1.1.

polecats are often found near human settlements (Elmeros et al., 2018, Sainsbury et al., 2024, and in this study), where the usage of multiple types of ARs against rats and mice, albeit in smaller quantities, is more common. This distinction is reflected in our results and supports our hypothesis: European polecats tend to contain a wider range of AR types, albeit in smaller quantities, while steppe polecats exhibit fewer types of AR accumulation but in higher concentrations. The lack of significant difference in concentrations between the two species can be attributed to the higher variability in the steppe polecats, which had both high and low concentration values, leading to a greater standard deviation compared to European polecats (Table 3). Another potential factor contributing to the differences is interspecific variations in prey selection. European polecats have a more diverse diet than steppe polecats, which includes a higher proportion of beetles, amphibians, eggs, and birds (Lanszki et al., 2019). This broader dietary niche may expose them to a wider array of ARs compared to the steppe polecat, which primarily preys on common hamsters and mainly consumes small mammals like common voles (Lanszki and Heltai, 2007). The fact that the common hamster is targeted with by ARs in Hungary (Cserkész et al., 2020) and most of the prey species of steppe polecat are also targeted with ARs can explain the higher AR concentrations found in steppe polecat. A third potential factor to consider is the different sensitivity of these species to toxic chemicals, as the dose-response relationship can vary between species (Rached et al., 2020). However, further studies are necessary to examine whether such differences exist between steppe polecats, European polecats and other mustelids.

The spatial distribution of the collected samples of the two polecat species is different and influenced by various factors. Firstly, the polecats' distinct habitat preferences have a significant influence. Carcasses of steppe polecats were predominantly found in agricultural areas, primarily within the Great Hungarian Plain (Southeast Hungary, especially in Békés County, and Little Hungarian Plain, especially Győr-Moson-Sopron (GYMS) County (Northwest Hungary), where the common hamster was abundant during the study period). The steppe polecat's range is primarily covering the eastern part of Hungary, where the European polecat is scarce. In contrast, European polecat samples were obtained from a more diverse range of habitats in Western Hungary, such as shrubland, wetlands, forests, settlements, and, in some instances, from higher altitudes.

The utilization of ARs may also vary across different regions of country. This variability may be linked to the unequal availability of specific types of ARs, differences in local pesticide use traditions, or could even be influenced by resistance to certain agents in specific areas (Sainsbury et al., 2018; Cerkvenik-Flajs et al., 2024).

4.3. Influencing factors of AR residue accumulation

NoAR exhibited a positive correlation with the concentration of ΣAR in our study, a pattern supported by an example found in the literature (Elmeros et al., 2018), which indicates a synergetic effect between the accumulation of AR types and concentrations.

Demographic variables affected only the steppe polecat. ARs tend to accumulate over time in steppe polecats, beginning from fetal development (Serieys et al., 2015), and continuing through neonatal exposure via lactating females (Gabriel et al., 2012). Studies found that the number of accumulated ARs and their concentrations tend to increase with age in European polecats (Ruiz-Suárez et al., 2016; Sainsbury et al., 2018) as well. We found controversial sexual differences in AR accumulation, the ΣAR and brodifacoum concentrations were higher in male steppe polecats, while bromadiolone was in females. The effect of sex is also controversial in the literature. In some cases, such as in stoats (McDonald et al., 1998; Murphy et al., 1998), caracals (*Caracal caracal*) (Serieys et al., 2019) and Eurasian otter (*Lutra lutra*) (Regnery et al., 2024) females exhibit higher AR exposure levels. However, we can find example of higher male exposure e.g., in fishers (Gabriel et al., 2015), but in most cases there is no sexual difference in AR exposure (Shore et al., 1996; Ruiz-Suárez et al., 2016; Koivisto et al., 2018). The accumulation of ARs as a sub-lethal effect can cause worse body conditions, as indicated by Sainsbury et al. (2018). In line with this, we observed a negative relationship between ΣAR and brodifacoum concentration and SMI in steppe polecats as in stoats and weasels, where ΣAR concentration was also negatively associated with poorer condition (Elmeros et al., 2011). However, while bromadiolone is correlated with poorer conditions in these species, this AR was associated with larger SMI in our study. The higher SMI was also positively associated with higher NoAR. Other studies suggested that good body condition can correlate with elevated contaminant burdens because lipophilic contaminants, like the ARs studied, can be connected with high-fat prey (Watt et al., 2005; Chong and Mai, 2019; Regnery et al., 2024). Moreover, most of our specimens were road-killed, which might suggest that individuals with higher SMI have a better chance of surviving AR exposure, while lower SMI might related to higher mortality.

We also observed a temporal effect: the concentration of bromadiolone decreased over time in steppe polecat, possible indicating a positive impact of regulatory restrictions. This contrasts with international trends, such as in fisher (Gabriel et al., 2015), bobcat (Serieys et al., 2015) and European polecat (Sainsbury et al., 2018), where AR exposure has increased over time. In some areas, AR use varies with rodent outbreaks rather than following a linear temporal trend (Fernandez-de-Simon et al., 2022). We did not find a temporal trend for the European polecat, possibly due to the smaller sample size. During the sampling period, fewer European polecat carcasses were found. Moreover, the frequency of finding was uneven between years; for example no road-killed European polecats were found between 2000 and 2015 (Ottlecz et al., 2024).

Notably, we identified more influential factors related to the spatial variables in both species. Steppe polecats found in areas containing more grasslands and wetlands appeared to be more protected against ARs than the more human-modified landscapes. This phenomenon may be attributed to lower AR use against agricultural pests or in case of wetland habitats heightened efforts in water protection to prevent filtration into bodies of water, such as fishing lakes. From these results, we assumed indirectly that the intensive usage of ARs against pests in arable lands, which is the main habitat type of steppe polecat (Appendix B), increases the risk of poisoning. It is known that intense land use (e.g., crop (Sainsbury et al., 2018) or Christmas tree production (Elmeros et al., 2018), animal husbandry (López-Perea et al., 2019), mining (Thomas et al., 2017) is usually associated with higher AR use. Moreover, the presence of more dense human populations near steppe polecat habitats resulted in higher brodifacoum concentrations, while near European polecat habitats, led to higher bromadiolone concentrations. This difference suggests that brodifacoum is probably used more in arable lands, while bromadiolone usage is more connected to human settlements. European polecat sample sites were closer to settlements, and this species is more commonly found in human-modified landscapes (Appendix B). The human influence on higher AR exposure has also been observed in other studies, such as higher human population density in stone marten and several other species (López-Perea et al., 2015; López-Perea et al., 2019) and in urban, more anthropized areas (Elmeros et al., 2018; Lohr, 2018; López-Perea et al., 2019; Musto et al., 2024). European polecats found in forests contained lower concentrations of ΣAR and brodifacoum, indicating less AR use in Hungarian forestry compared to other forest types, e.g., public forests (Gabriel et al., 2012), industrially used boreal forests (Thomas et al., 2017) or Christmas tree production areas (Elmeros et al., 2018). The lower brodifacoum concentration in grasslands also suggests that AR exposure is lower in more natural habitats for both European and steppe polecats, similar to other species like bobcats (Serieys et al., 2015). In European polecats, which inhabit more heterogeneous habitats (Appendix B), spatial variables impact AR accumulation. In contrast, steppe polecats, living in more homogeneous habitats (Appendix B), are more affected by demographic factors in addition to spatial variables.

4.4. The potential lethality of accumulated ARs

In our study, we measured AR concentrations between 0.02 and 0.57 mg/kg, with the highest concentrations associated with brodifacoum. The concentration of accumulated toxicants in the examined individuals was often found to be at levels that could have resulted in severe AR exposure in the case of European polecats, which can be lethal as reported by Elmeros et al. (2018). Establishing a direct link between laboratory results and the cause of death can be challenging, because the toxicity of a specific substance is often unknown for a given species (Berny, 2007). One reason for this is that LD50 cannot be tested on wild species under laboratory conditions due to ethical and conservation reasons. Studies indicate that concentrations of 0.2 mg/kg or higher in ARs can be potentially lethal to mustelids (Grolleau et al., 1989; Newton et al., 1999, as cited in Elmeros et al., 2011 and Elmeros et al., 2011; Baert et al., 2015; Elmeros et al., 2018; López-Perea et al., 2019). There have been some instances where the cause of death due to secondary poisoning was clearly identifiable. For example, a European polecat found dead in a barn with 1.4 mg/kg accumulated difenacoum displayed characteristic haemorrhagic symptoms of AR accumulation, confirming secondary poisoning as the cause of death (Birks, 1998; Shore et al., 2003). These haemorrhagic symptoms can also manifest at lower concentrations, as evidenced by ferrets displaying symptoms at 0.6 mg/kg bromadiolone (Fournier-Chambrillon et al., 2004). However, moderately high AR concentration values, as in our case, are not necessarily associated with visible haemorrhagic symptoms at necropsy, because most of our collected polecat specimens were road-killed, so this trauma precluded clinical detection of any haemorrhaging related to ARs (as in other studies as well (Sainsbury et al., 2018)). It is essential to exercise caution when drawing conclusions, as the presence of AR residues alone is insufficient to infer lethal poisonings (Sainsbury et al., 2018). In those studies where road-killed polecats were examined as in our study, secondary poisoning was not the direct cause of death. Nonetheless, it is possible that the accumulated AR residues contributed to the occurrence of the direct cause of death through sub-lethal effects (Shore et al., 1996; Sainsbury et al., 2018; Van den Brink et al., 2018; Sainsbury et al., 2020). The sub-lethal effects can be related to many factors, for example susceptibility to diseases (Serieys et al., 2015; Elmeros et al., 2018; (Fraser et al., 2018; Serieys et al., 2018; Carrera et al., 2024) and behavioral changes, e.g., movement coordination problems (Knobel, 2015). Individuals weakened by canine distemper virus (CDV) are prone to hunt available prey with higher exposure of ARs or are more sensitive to the effects of lower concentrations of ARs (Carrera et al., 2024). In our study, two steppe polecats (28001, 28037) were found to be infected with CDV (Lanszki et al., 2022) and concurrently exhibited the presence of ARs in low concentrations (0.08 mg/kg and 0.02 mg/kg) from them. Sub-lethal effects of AR residues can manifest months after exposure to ARs (Shore et al., 1999). Additionally, these could appear as a hidden, negative effect on population dynamics through e.g., different effects, maybe different mortality of sexes, or sublethal effects, which can cause several disadvantageous consequences such as earlier mortality. However further research is needed to substantiate these theories.

4.5. Nature conservation and practice

Our results clearly demonstrate that the use of ARs poses a significant threat to polecat populations through the process of bioaccumulation. These ARs also pose a threat to the whole ecosystem from the primary consumers through secondary consumers such as polecats (and presumably other mesopredators) to the tertiary consumers of polecats which play an important role in the associations as top predators, e.g., the strictly protected Eastern imperial eagle (*Aquila heliaca*) (Horváth et al., 2018) and red kite (*Milvus milvus*) (Mougeot et al., 2011). These ARs are persistent, stay in the environment and the food web (Kotthoff et al., 2019) for a long time and can cause poisonings long after the complete prohibition of use, e.g., as DDT (Kabasenche and Skinner, 2014). When considering the conservation of polecats, it is imperative to recognize this threat as a critical issue that demands attention and action. The initial measure to mitigate secondary poisonings involves reducing primary poisonings, serving as the foundational step for comprehensive prevention. This approach aligns with ethical rodent control principles (Meerburg et al., 2008), which prioritize practical solutions that consider animal welfare and humane actions alongside efficiency and economy. Key strategies include prevention, effective habitat management (Meerburg et al., 2008; Connolly et al., 2009), rodent relocation (Meerburg et al., 2008; Schell et al., 2021), using biological control methods (Paz et al., 2013; Fox, 2014) and financial incentives (Jackson and Wangchuk, 2001; Haney, 2007; La Haye et al., 2010; Surov et al., 2016; Schoukens, 2017). Preventing poisoning can be aided by official crime prevention measures, quality public education, setting positive examples in families, shaping attitudes through media, and overall, increasing environmental awareness among residents. Establishing a robust control system in Hungary that includes a national registry of the types and quantities of toxic substances purchased and deployed in specific areas is crucial. This collaborative effort aligns with the current aims of the European Union, and can be facilitated by scientific institutions or natural history museums (Movalli et al., 2022). Less active human engagement in rodent control and support ecosystem services instead, e.g., protecting predators of rodents as polecats or reuse and manage properly the organic waste thereby reduce the overgrowth reproduction of rats would help in set a natural balance. While a complete prohibition of the use of secondary poisoning-causing chemicals would be the ideal solution to this nature conservation problem, even a reduction in their use and more responsible application would offer numerous benefits to the natural world, which would also have a positive effect on human health (Zinsstag et al., 2011). It is essential to recognize the urgency of addressing this issue and take proactive measures to protect polecat populations and the broader ecosystem.

Careful consideration of future measures is crucial during the planning stage, as several examples have demonstrated that the actual restrictions or measures are not effective enough to protect wildlife (Regnery et al., 2024). According to our study this is also the case in Hungary: there were still documented poisoning cases after the regulatory restrictions came into force (Deák et al., 2020; Deák et al., 2021). Even our polecat samples from 2021 contained accumulated ARs despite regulatory restrictions implemented in 2017 prohibiting the use of coumarin-type rodenticides in arable lands and other open areas. However, the regulations state that the usage of these examined six coumarin-type ARs is prohibited in open areas and in high concentrations (it cannot be defined exactly and we also did not collect data from the field). There can be several reasons for the failure of these restrictions. One main reason is non-compliance with regulatory restrictions. The practice of illegal poisoning is quite frequent in Hungary. For example, the use of carbofuran has been prohibited since 2008, yet lethal poisonings have been common since then (Deák et al., 2021). We have an example of illegal activity (Supplementary material 2) in relation to the examined ARs: we have photo documentation of rodenticide products applied on the surface, which contained bromadiolone, near Kunágota (Supplementary material 2, Fig. S2).

5. Conclusion

This study represents the first comprehensive examination of toxic chemical substances in Hungary and Central Europe in the European polecat, and it is the first global evidence of secondary exposure in the steppe polecat. The level of AR accumulation in our samples was comparatively lower than observed in most European countries. However, it remains a significant concern that demands attention, irrespective of the scale. The fact that almost half of the examined individuals were found to be exposed to ARs underscores the need for ongoing and systematic monitoring of poisoning cases in Hungary. Furthermore, AR accumulation poses a significant threat to polecat species, emphasizing the importance of monitoring in other countries within their distribution range. In light of our new results, it is urgent to reconsider the conservation management strategies for species threatened by AR exposure, thereby secondary poisoning. It is essential to transition our current agricultural practices towards a more environmentally conscious approach that prioritizes the protection of wildlife and ecosystems.

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CRediT authorship contribution statement

Julianna Szulamit Szapu: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tamás Cserkész:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Zsolt Pirger:** Writing – review & editing, Validation, Methodology. **Csaba Kiss:** Formal analysis. **József Lanszki:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jozsef Lanszki reports was provided by HUN-REN Balaton Limnological Research Institute. Zsolt Pirger reports was provided by HUN-REN Balaton Limnological Research Institute. József Lanszki reports a relationship with HUN-REN Balaton Limnological Research Institute that includes: employment. Zsolt Pirger reports a relationship with HUN-REN Balaton Limnological Research Institute that includes: employment. József Lanszki and Zsolt Pirger was supported by the RRF-2.3.1-21-2022-00014 (National Multidisciplinary Laboratory for Climate Change), the RRF 2.3.1-21-2022-00008, BLRI (National Laboratory for Water Science and Water Security) and the NP2022-II3/2022; BLRI (Sustainable Development and Technologies National Programme of the Hungarian Academy of Sciences). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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